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by

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ABSTRACT

DRINKING WATER AND LEGIONNAIRES' DISEASE*

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Pneumonia outbreaks caused by Legionella species recently have been epidemiologically linked to potable water distribution systems in hospitals and hotels. Showerheads were confirmed as the immediate source of the Legionella in many of the outbreaks, however, the organism also was isolated from other parts of the water distribution systems, such as water taps and storage tanks. Legionella also have been isolated from building water distribution systems in the absence of the disease and this has led to questions about the need to disinfect all systems in which the organism is found. In disease outbreak situations, where the requirement for disinfection is appropriate, chlorination appears to be the method of choice. Chlorine "shock" treatment (50 mg/L free chlorine for short periods) followed by continuous chlorination (1-2 mg/L free chlorine) was effective for eliminating Legionella from plumbing systems. High temperature (60°C for 72 hours) was partially effective for removing most of the Legionella for 1 to 2 month periods. Ozone and ultraviolet irradiation were not effective. The role of potable water as a vehicle for the transmission of Legionella species is ambiguous, since the organisms have not been isolated from mains delivering water to buildings whose potable water plumbing systems have yielded the organism. Recommendations for the control of Legionella that are consistent with the current state of our knowledge of this pathogen are discussed.

*This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

Introduction

In 1976 an outbreak of acute respiratory illness developed in members of the Pennsylvania American Legion who had attended a state convention in Philadelphia. Approximately 7 percent of the attendees became ill (1) and there was a 15 percent mortality rate among cases (2). Laboratory investigators were unable to discover the cause of the disease until six months after the start of the outbreak. At that time workers from the Centers for Disease Control isolated and characterized a bacterium which was later named Legionella pneumophila. The illness was initially given the name Legionnaires' Disease (LD), but currently it is called legionellosis.

Most of the early outbreaks of legionellosis were linked epidemiologically to cooling towers and evaporative condensers attached to buildings such as hotels (2,3,4), hospitals (5,6), a shopping mall (7), and even a golf clubhouse (8). More recently a number of reports have described outbreaks of legionellosis associated with potable water. It is the intent of this review to examine the relationship between drinking water and legionellosis and determine, if possible, whether there is a need and a means for controlling this pathogen in potable water systems.

Clinical Disease

Legionellosis is currently recognized in two distinct forms which are summarized in Table 1. Legionnaires' Disease is characterized by a pneumonia associated with significant mortality rates whereas the second

form, called "Pontiac fever" is a non-pneumonic, self-limiting febrile illness with a very high attack rate. The incubation period for Legionnaires' Disease is 2 to 20 days while for Pontiac fever it is only 5 to 66 hours. It is interesting to note that, even though the disease is respiratory in nature, person-to-person transmission of legionellosis has not been conclusively established.

Isolation and Detection Methods

Legionella pneumophila is a gram-negative, slow growing, rod-shaped bacterium which does not grow on ordinary laboratory media. The fastidious nature of this pathogen contributed in part to the difficulty encountered in developing a bacteriological medium for recovering the organism from environmental water samples. Several media (9,10,11,12,13) recently have been developed which appear to be very promising but they have not had enough use under varied conditions to be properly evaluated. The lack of an efficient isolation medium at the time of the Philadelphia outbreak led to the development of a direct immunofluorescent (DFA) test for diagnosing legionellosis (14). The DFA reagent is prepared by immunizing rabbits with suspensions of formalin killed Legionella cells. Gamma globulin is extracted from the antisera and conjugated to fluorescein-isothiocyanate. The DFA reagent is flooded over air dried, heat fixed smears of material suspected of containing Legionella (Figure 1). Stained smears are then examined with a fluorescent microscope. The method can be used to quantify the number of fluorescing cells by placing a known volume of material on a special slide and then counting a pre-selected number of fields whose total

area represents some fraction of the volume of material placed on the slide. The DFA test is a rapid procedure for detecting Legionella but it has the disadvantage of being unable to determine if the detected cells are alive or dead. This deficiency may be overcome by intraperitoneal inoculation of suspected material into guinea pigs (Figure 2). The animals develop febrile illness within two days and usually become moribund in six days if the sample is positive. The peritoneal exudate, spleen and liver tissues of animals killed a few days after injection usually contain high densities of bacteria. Homogenates of lung, liver, and spleen tissue are then customarily inoculated into embryonated hens' eggs. Death of the embryos occurs in 4 to 7 days. Yolk sac smears are then examined for the presence of Legionella and bacteriological media are inoculated with yolk sac tissue.

The DFA test and animal isolation procedures were developed for clinical specimens but were soon adapted for use with environmental samples. However, the low densities of Legionella found in environmental samples required concentration of the samples. This has customarily been accomplished by centrifugation whereby sample volumes as large as 20 liters are spun down and the sedimented cells are resuspended in small workable volumes. A flow chart of the procedure for environmental samples is shown in Figure 3.

There are a number of recognized species of Legionella other than L. pneumophila. Legionella micdadei (15), L. bozemanii (16), L. dumoffii (16), and L. longbeachae (17) are four new species that also have been

implicated as the cause of human pneumonia. Two other new species, L. gormanii (18) and L. jordanis (19), have been isolated from the environment. These latter two species have not been shown to be the cause of human pneumonia, but they have been implicated serologically as possible pathogens associated with unrecognized human infections. L. pneumophila and other species also have been differentiated further using serological methods. Antibodies produced against killed Legionella cells have been used to characterize distinct serogroups within the species. Six serogroups of L. pneumophila have been characterized to date. Although each of the other species has only one designated serogroup, it is likely that more will be found in the future.

Sources of Legionella

Surface Waters - In 1978, shortly after an outbreak of legionellosis in Bloomington, Indiana, water samples from an apparently thermally polluted stream near the focus of the outbreak were examined for the presence of Legionella pneumophila (20). Three of the samples were found to be positive by the guinea pig isolation procedure. This isolation of Legionella from surface water was followed by many other isolations from aquatic environments not associated with disease outbreaks. Fliermans et al (21) screened 200 water samples from 23 lakes in Georgia and South Carolina using the DFA technique. Over 90% of the samples contained fluorescing cells. Four of the samples were inoculated into guinea pigs to confirm the presence of viable Legionella and all were found to be positive. The concentration of Legionella in the lake samples confirmed as containing viable organisms ranged from 10^5 to 10^6 cells per liter. Much

later Fliermans et al (22) expanded their survey to include the states of North Carolina, Florida, Alabama, Illinois, and Indiana. Seven hundred and ninety-three samples from 67 different lakes were almost all positive by the DFA test. Guinea pigs were inoculated with concentrates from 318 of the water samples from which 47 (15%) strains of Legionella were isolated. Bacterial densities calculated from the DFA enumeration ranged from the limit of sensitivity, 9×10^3 per liter, to an upper limit of 3.3×10^7 per liter. Legionella have also been isolated from pristine, as well as, contaminated, waters in Puerto Rico (23). Densities ranged from 1×10^3 to 1×10^5 per ml in both environments. The finding of Legionella pneumophila in these widespread areas would seem to indicate that this organism may be ubiquitous in aquatic environments.

Potable Waters - Legionella have been associated with potable water in a number of instances. A summary of the isolations from potable water sources is shown in Table 2. The high isolation frequency in hospitals may be deceptive since in many cases surveys were conducted in response to outbreaks of legionellosis. In fact, most of the isolations were obtained in hospitals where epidemiological evidence indicated that showerheads might be the source of the pathogens. Three hospitals in the United States were among the first to detect L. pneumophila in showerhead samples using guinea pig isolation techniques (24). Similar results were obtained in England where positive isolations were obtained not only from showerhead samples, but also from water storage tanks, and hot and cold water taps (25,26,27). It is interesting to note that in two of the studies, samples

from the main water supply line were negative with the guinea pig test (26,27). This finding does not necessarily imply that the water samples did not contain Legionella but rather that the sample may not have contained a high enough density of organisms to cause an infection in the test animal. The presence of low densities of Legionella in water mains also is implied from studies wherein the organism appeared very soon after successful chlorination procedures had been halted. This also suggests that water storage tanks, water taps, and showerheads may provide a suitable environment for the growth of Legionella. Tison and Seidler (28) found DFA positive samples in raw and treated drinking water from systems receiving four different types of treatment. Samples that contained 10,000 Legionella per liter or greater, as calculated from the DFA procedure, were inoculated into guinea pigs. Although over half of the DFA positive samples contained 10^4 or more Legionella per liter, none of them elicited a positive response in guinea pigs. The authors speculated that the DFA positive cells may not have been viable, or that they were viable but avirulent or that they were not able to survive the isolation procedures. Stout et al (29) used a selective medium to isolate L. pneumophila from 9 of 15 shower water samples and 23 of 25 faucet water samples. Fifty more sites, including showerheads, faucets and water storage areas, were studied later by the same group of investigators (30). Two of the sites were positive for Pittsburgh Pneumonia Agent (L. micdadei) and 33 were postive for Legionella. It might appear that Legionella can be found only in hospital environments, however, such is not the case. Tobin and his colleagues (25) found 3 out of 31 water samples from showerheads, water taps and water tanks positive for Legionella from hotels not

associated with known legionellosis cases. They also found the organism in 3 out of 3 water samples from hotels associated with Legionnaires' Disease. Similar results were observed in Italy where shower water samples from a hotel associated with a case of legionellosis were positive in guinea pigs (31).

Health Significance of Legionella in Drinking Water

A summary of the number of outbreaks and cases of legionellosis associated with potable water that have occurred since 1979 is shown in Table 3. It is obvious that the outbreaks reported in the literature have been few in number and this may lead to the conclusion that the health significance of Legionella in drinking water is negligible. This might be an inappropriate conclusion at the present time. A study of these outbreaks may lead to a better understanding of the causes of sporadic cases and of the very high seropositive frequency found in some segments of the population (see Table 4). Most of the outbreaks of legionellosis linked to showerheads or water taps have occurred in hospital environments and this may lead to the conclusion that Legionella pneumophila is an opportunistic pathogen, which by definition requires an individual who is compromised in some manner, i.e., receiving immunosuppressive drugs or having recently been subjected to traumatic surgical procedures, such as the transplant of an organ. This appeared to be true at the Northwestern University Medical Center (24) and also in the cases observed in Oxford, England (32) where L. pneumophila, serologically identical to isolates obtained from renal transplant patients who had contracted legionellosis, were isolated from showerhead water samples. Eight of 12 patients in the

Kingston Hospital in Surrey, England who had developed pneumonia caused by L. pneumophila were either immunosuppressed or debilitated (27). However, the remaining four were healthy adults, which somewhat tempered the conclusion that Legionella infections were opportunistic in nature. Since the plumbing in the Kingston Hospital was extensively contaminated with Legionella, it was speculated that the density of the organism might be a critical factor. The conclusion that compromised individuals are a necessary element for the development of legionellosis is further weakened by potable water associated outbreaks that have occurred in hotels, especially those without heat exchange systems.

In 1980 an unusual number of Legionella pneumonia cases in a hospital in Como, Italy led to a search for the source of these infections (33). A review of hospital records indicated that 12 persons who had been hospitalized with pneumonia all had stayed at one hotel. A survey of 94 individuals from two tour groups who had stayed at the hotel just prior to its closing for the season revealed that 22 percent had a febrile illness within two weeks of departure from the hotel. Forty-three percent of those ill had been hospitalized and two had died. Water from several showers and sinks in guest rooms at the hotel were DFA positive for L. pneumophila. Subsequent examination of water samples from the hotel for viable Legionella using guinea pigs showed them to be positive for the same serogroup that gave high antibody titers in the sera of legionellosis patients. Another group of legionellosis cases associated epidemiologically with a hotel was reported in England (26). The onset of pneumonia in four members of a golfing party appeared to be related to an

overnight stay at a hotel. A retrospective search of the records revealed that four other individuals who had stayed at the hotel also had developed pneumonia either during their stay or shortly afterward. L. pneumophila was found in the hotel's water storage tanks, hot and cold taps, and in the shower outlets. The water sample isolates were serogroup 1, the same antigen type which elicited a positive response in the sera of three of the individuals with legionellosis.

The link with potable water is best illustrated by the Kingston Hospital outbreak (27). Epidemiological evidence pointed to the hot and cold water taps as the common source for some of the cases. The dramatic decrease in the number of cases after disinfecting the water delivery system considerably strengthened the implied association. The legionellosis rate dropped from 2.5 to 0 per 1000 admissions after chlorine application (1-2 ppm free chlorine) and raising the hot water temperature to 55°-60°C while other respiratory infection rates remained stable or actually increased.

Control of Legionella in Potable Water Systems

Free chlorine has been found to be an effective agent for the control of Legionella in potable water. Skaliy et al (34) showed that 1.3×10^6 viable Legionella cells were inactivated almost immediately after exposure to 3.3 and 6.6 mg per liter of free chlorine. Although the chlorine levels used in this laboratory study were unusually high, the effectiveness of this disinfectant has been confirmed under actual conditions in the Kingston Hospital outbreak (27). The control of Legionella in the water

system was accomplished by maintaining 1-2 parts per million (ppm) free chlorine at all outlets. In the hot water system only 0.2 ppm free chlorine could be maintained and this was not sufficient to eliminate L. pneumophila from the system. The hot water temperature was only 45°C, hot enough to drive off chlorine but not deleterious to Legionella. The temperature was increased to between 55° and 60°C, a procedure that eliminated L. pneumophila from the hot water system.

Chlorine levels must be maintained within a system, otherwise the organism will reappear. This was shown by Tobin et al (32) in their attempt to eradicate Legionella from a hospital water system. The procedure they used was as follows: Free chlorine at 50 ppm was maintained in the tank feeding the cold water system for three hours. The tank was drained and then refilled with chlorinated water (50 ppm) which was subsequently run into the cold water system and held there for three hours before the system was flushed with non-chlorinated water. The hot water system was treated similarly. This "shock" chlorine treatment eliminated L. pneumophila from the system for up to six months. However, when the water was examined at 9 months, the organism was found again. The "shock" chlorination treatment also was used at a hotel where four cases of legionellosis had occurred (26). Fifty ppm free chlorine was used to eradicate L. pneumophila from the water distribution system. After this treatment, a continuous chlorination unit was put into operation (chlorine concentration not given). No cases of legionellosis occurred at the hotel in the nine months following the start of chlorination. Intermittent high temperature treatment of a water system without chlorination also appears to be an

effective short-term means for controlling the level of Legionella in a hospital hot water distribution system (35). The hot water temperature was increased from 45°C to 60°C for 72 hours after which the entire system was flushed for 20 minutes. The number of positive samples decreased by 80 percent within 72 hours and remained at approximately this level for two months after which the process had to be repeated. Ozone treatment of a hospital building water system contaminated with Legionella does not appear to be an efficient means of eliminating the organism (36). Neither high level-short term (1 mg/L, 3 days) nor long term-low level (0.5 mg/L, 21 days) ozone treatment was effective in removing Legionella from the system.

Although the effectiveness of ultraviolet radiation for inactivating Legionella in drinking water systems has not been reported, a laboratory study (37) has shown reduction of 90 to 99% at doses of 920 to 2,760 $\mu W\text{-}s/cm^2$, respectively. This study indicated that Legionella pneumophila was more sensitive to inactivation by ultraviolet irradiation than Escherichia coli, Salmonella typhi, Serratia marcescens, and Pseudomonas aeruginosa for which doses of 2,110 to 5,500 $\mu W\text{-}s/cm^2$ were required to produce 90% inactivation.

A summary of the above control measures is given in Table 5.

Over view

The available data on the association of Legionella pneumophila with

drinking water has established a discernible pattern which may prove to be useful for making an intelligent assessment as to how we should deal with this organism. This pattern is shown schematically in Figure 4. Its primary source appears to be the aquatic environment. The organism has been found in association with blue-green algae (38) which may provide essential nutrients for Legionella in aquatic habitats that otherwise would not support rapid growth. The association with algae has been used to suggest an explanation for the apparent widespread distribution of the organism in the environment. The enhanced survival of aerosolized Legionella suspended in algal extracts has also been reported (39) and this finding is compatible with the cooling tower-associated outbreaks. The widespread occurrence of outbreaks of legionellosis indicates that the organism is probably not indigenous to any one area of the United States, or the world for that matter, although most reports have come from western Europe, Britain and North America. It does not grow to high densities under natural conditions as indicated by studies on its ecological distribution where 33,000 organisms per ml was the highest density observed (17). In raw water sources and distribution systems, densities as high as 100 per ml have been observed using the DFA techniques (28), however, the Legionella could not be isolated using guinea pig inoculation. This finding was similar to those in hospitals where outbreaks of potable water associated legionellosis occurred and viable organisms could not be detected in water samples from the main water source entering the hospitals. Isolation of Legionella in these same establishments frequently has been accomplished with water samples from showerheads, faucets and water storage tanks, especially from hot water distribution systems.

Legionella pneumophila appears to have the ability to colonize and grow in certain parts of water distribution systems in buildings, under what are as yet undefined conditions, although warm water certainly seems to enhance this ability. Legionella have been found to grow in certain species of free living freshwater and soil amoebae (40). These amoebic species have been isolated from humidifiers, and, if they are inhabitants of slime in showerheads, may offer an explanation for the presence of large numbers of Legionella in samples from such fixtures. It has been estimated (40) that a person inhaling a single infected amoeba could receive a dose of up to 1,000 Legionella cells. Epidemiological evidence indicates that aerosolization of the potable water is a necessary element in the infection process, since most cases observed to date seem to have contaminated showerheads as a common factor. This observation is not unexpected, since aerosols from cooling towers have been implicated in many outbreaks (2,3,4) and, furthermore, it is known that Legionella can be concentrated in aerosol droplets by factors of 100 to 10,000 relative to their concentrations in bulk water (41).

The propensity of this organism to infect compromised individuals gives the impression that the organism is an opportunist whose victims are found mainly in hospitals. Some of the evidence available indicates that this may not be true since apparently healthy individuals in hospitals have developed legionellosis that has been linked to drinking water. Furthermore, outbreaks have occurred among healthy guests at hotels without cooling systems, where Legionella has been isolated from the water distribution systems. Most of the outbreaks attributed to cooling towers

or evaporative condensers have occurred in healthy persons and although it has not been shown, it is quite possible that the source for Legionella found in these heat exchange systems may have been the make-up water drawn from the potable water distribution system. This emerging pattern of the association of drinking water with legionellosis indicates that potable water may be merely the vehicle for transporting this newly recognized pathogen from its customary niche in nature to a man-made habitat, where it is able to survive and flourish. Moreover, it appears that it is not the primary, but rather the secondary, use of potable water, such as for showering, that is a risk factor for legionellosis. Experimental and epidemiologic evidence favors the airborne route of infection. Although the route of entry of the organism may be respiratory, systemic spread to the lymph nodes, liver, spleen, heart, and kidney has been reported (42). There has been only one report of an attempt to infect animals orally with Legionella administered in drinking water. Conner and Gilbert (43) administered up to 1×10^8 bacteria per day in drinking water to each of three guinea pigs for seven days and found no evidence of infection or disease. In contrast, the aerosol infective dose in guinea pigs has been shown to be less than 129 organisms (44). In another study (45), guinea pigs infected by nasal instillation did not develop disease but animals infected by the aerosol route did. Although the available evidence clearly indicates an airborne route of infection, the source of the organism in distribution system fixtures, and the conditions that result in converting a potential hazard into an actual hazard, have not been established.

Recommendations

Not long after it was conclusively shown that some of the major outbreaks of legionellosis were associated with cooling towers and evaporative condensers and that a cumbersome, but relatively easy method (DFA test), was available for examining water samples, there was a widespread demand for information on the control of this organism. It seems that everyone who had a DFA test available was finding Legionella in heat exchange system water samples and wanted to do something about it. Recommendations were solicited from federal agencies such as the Centers for Disease Control and the U.S. Environmental Protection Agency as to the proper biocide to use. However, it soon became apparent that the organism could easily be found in cooling system water in the absence of legionellosis and the rush to disinfect subsided. The state of our knowledge about the relationship of Legionella to drinking water is at a point similar to our knowledge about the relationship of this organism to heat exchange systems in 1977. It is appropriate at this time to gather health effects information and evaluate the risk to the population from drinking waterborne Legionella before recommending specific measures that may be unnecessary, i.e., elimination of the organism from the water mains. However, there is justification for suggesting certain guidelines that may be appropriate in situations where the occurrence of Legionella pneumophila in potable water presents a health hazard. In late 1981, a microbiology workshop on revisions to the National Interim Primary Drinking Water Regulations, sponsored by the EPA Office of Drinking Water

in conjunction with the American Society for Microbiology, considered some of the problems presented by the occurrence of opportunistic pathogens in drinking water. A portion of the draft guidelines that evolved from the group considering these pathogens dealt exclusively with Legionella pneumophila and these suggestions are presented here. It was recommended that:

1. Institutions with high risk populations, such as those found in hospitals and nursing homes, should be made aware of the potential health hazards associated with the presence of Legionella in finished water.

2. Water supplies with large holding reservoirs should be informed that Legionella can grow in these storage systems.

3. Legionella pneumophila in the piping systems of new public use facilities such as hospitals, hotels and work places should be controlled by disinfection according to the recommended AWWA procedures for new pipes.

The above recommendations are in draft form and are subject to revision. Considering the present state-of-knowledge, the recommendation of Tobin, Swann, and Bartlett (19) appears to be appropriate. These investigators suggested that the demonstration of L. pneumophila in water systems in the absence of cases should not result in the implementation of dubious eradication methods. Specific recommendations for efficacious control can only be made when more information on the occurrence and distribution of the organism in water supplies becomes available, and when the extent of the hazard from this route of exposure has been evaluated.

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TABLE 1

FORMS OF LEGIONELLOSIS

Illness Type	Incubation Time	Attack Rate	Mortality	Symptoms
Legionnaires' Disease (pulmonary)	2-10 days	0.1-0.5%	16%	fever malaise chills cough rales lung consolidation pneumonia
Pontiac Fever (non-pulmonary)	5-66 hours	95%	none	malaise myalgia fever chills headache flu-like

TABLE 2

LEGIONELLA ASSOCIATED WITH POTABLE WATER TREATMENT PLANT SYSTEMS

Environment	DFA Detection		Isolation	
	No. Samples	No. Pos.	No. Samples	No. Pos.
Hospitals[1,2]			195	72
Hotels[1,3]	2	2	35	7
Water Treatment Plant[4]	28	28	15	0

[1]Showerhead, faucet and storage tank samples

[2]References 24, 25, 27, 29, and 31

[3]References 25 and 31

[4]Reference 28

TABLE 3

POTABLE WATER-ASSOCIATED LEGIONELLOSIS[1]

Environment	No. Outbreaks	No. Cases
Hospitals[2]	5	135
Hotels[3]	3	60

[1]Does not include outbreaks attributed to cooling towers

[2]References 24, 27, and 32

[3]References 26, 31, and 46

TABLE 4

PREVALENCE OF ANTIBODY TO
LEGIONELLA PNEUMOPHILA IN SELECTED POPULATIONS

Group	No. Tested	No. Positive	% Positive	Ref.
Indoor/Outdoor Workers (Columbus, OH)	588	117	19	47
Industrial Workers (Burlington, VT)	110	17	15	48
High Risk Workers	21	4	19	49
Low Risk Workers (New York, N.Y.)	27	6	22.2	49
Wastewater and Utility Workers (Chicago, Cincinnati, Memphis)	509	48	9.4	50
General Population ≥46 years old	1143	19	1.7	51
General Population Age 50-104	1000	53	5.3	52
Patients (non-legionellosis)	713	24	3.4	53
Hotel Guests	N.G.*	N.G.	17	54

*Not Given

TABLE 5

CONTROL MEASURES FOR *LEGIONELLA* IN WATER PLUMBING SYSTEMS

	Treatment	Treatment Frequency	Effective	Ref.
Chlorine	5 mg/L "shock" 1-2 mg/L continuous	Continuous	Yes	27,32
High Temperature	60°C - 72 hours (flush)	1-2 mos.	Partially	35
Ozone	1 mg/L (3 days) 0.5 mg/L (21 days)	--	No	36
UV Radiation	1840-2760 $\mu W\text{-}s/cm^2$	Continuous	--	37

Figure 1. Schematic diagram of the direct fluorescent antibody procedure for detecting Legionella species.

Figure 2. Schematic diagram of the procedure for determining Legionella viability using the guinea pig and embryonated hen's egg.

Figure 3. Flow diagram of procedure for concentration and isolating Legionella from water samples.

Figure 4. Schematic diagram of the hypothetical relationship of Legionella to water distribution system.

Figure 1.

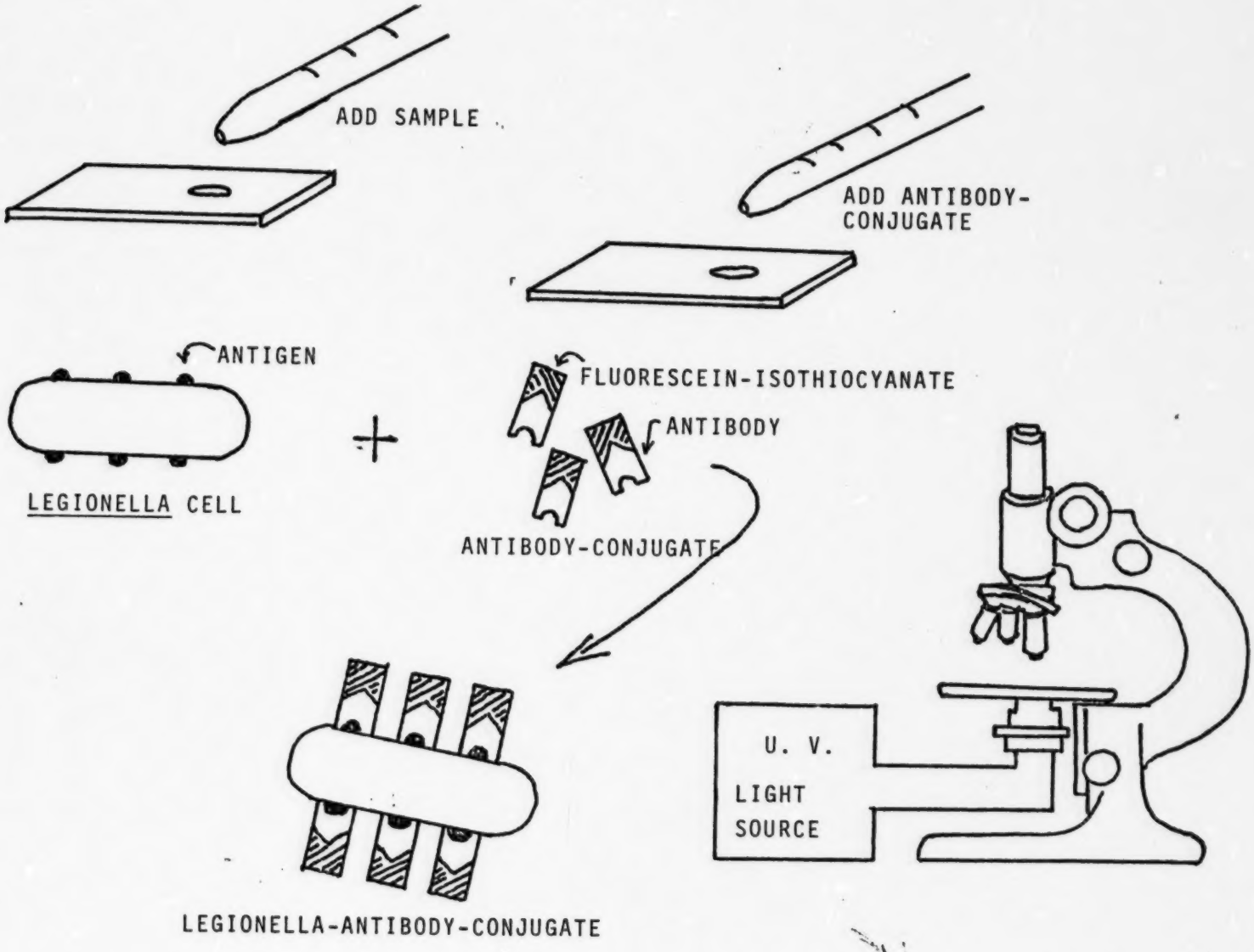

ADD SAMPLE
ADD ANTIBODY-
CONJUGATE
ANTIGEN
FLUORESCEIN-ISOTHIOCYANATE
ANTIBODY
LEGIONELLA CELL
ANTIBODY-CONJUGATE
U. V.
LIGHT
SOURCE
LEGIONELLA-ANTIBODY-CONJUGATE

Figure 2.

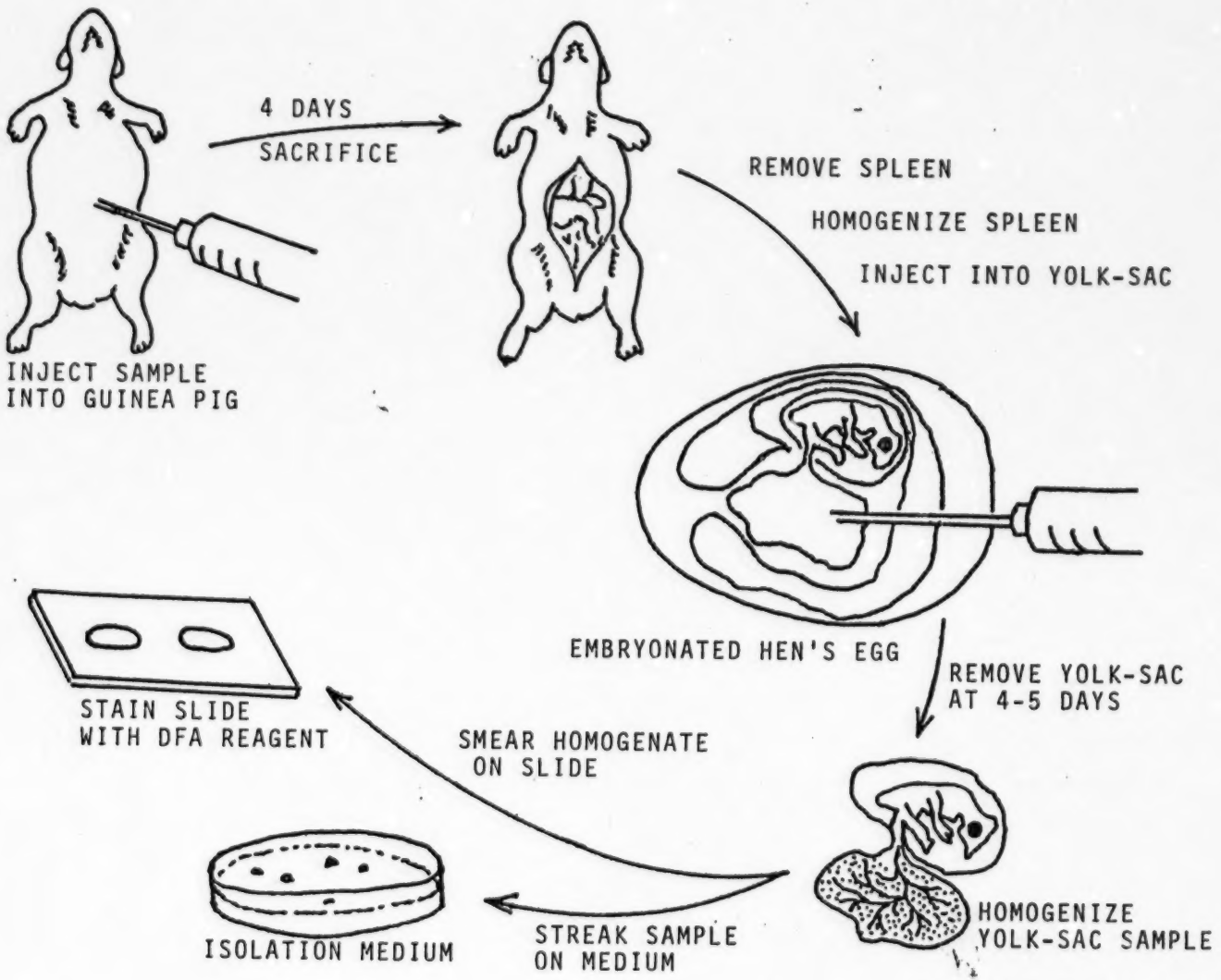

4 DAYS
SACRIFICE
INJECT SAMPLE
INTO GUINEA PIG
REMOVE SPLEEN
HOMOGENIZE SPLEEN
INJECT INTO YOLK-SAC
EMBRYONATED HEN'S EGG
REMOVE YOLK-SAC
AT 4-5 DAYS
HOMOGENIZE
YOLK-SAC SAMPLE
STREAK SAMPLE
ON MEDIUM
ISOLATION MEDIUM
SMEAR HOMOGENATE
ON SLIDE
STAIN SLIDE
WITH DFA REAGENT

Figure 3.

FLOW CHART FOR LEGIONELLA ISOLATION

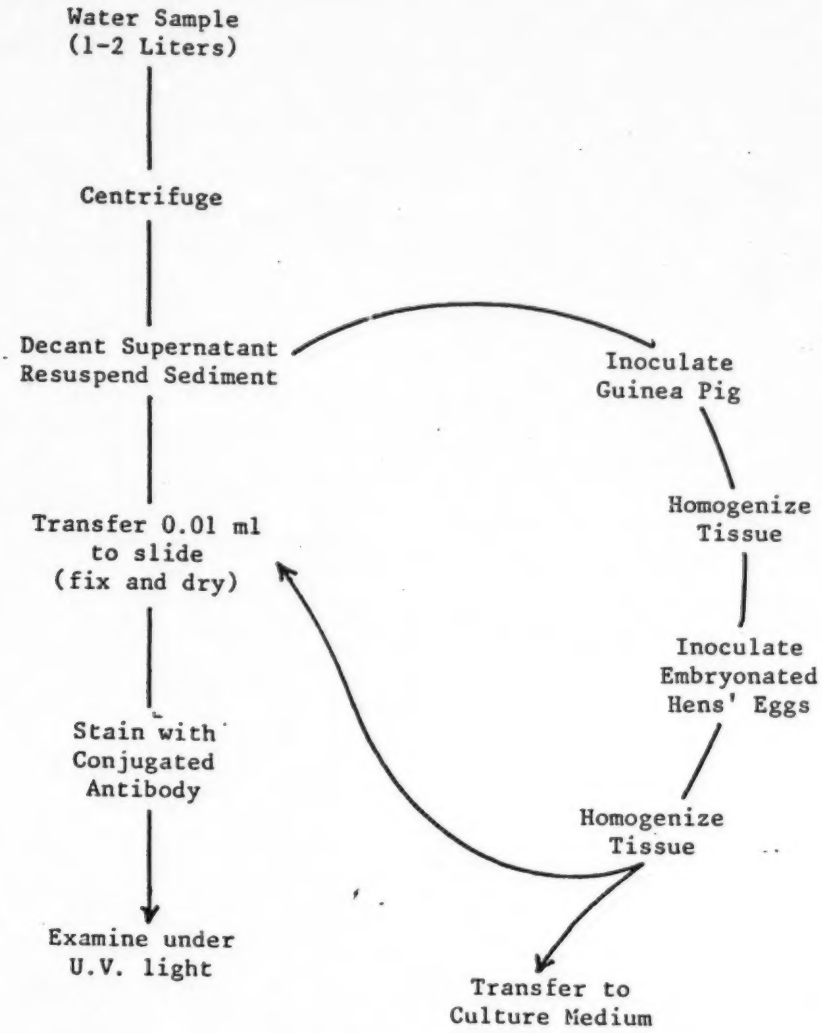
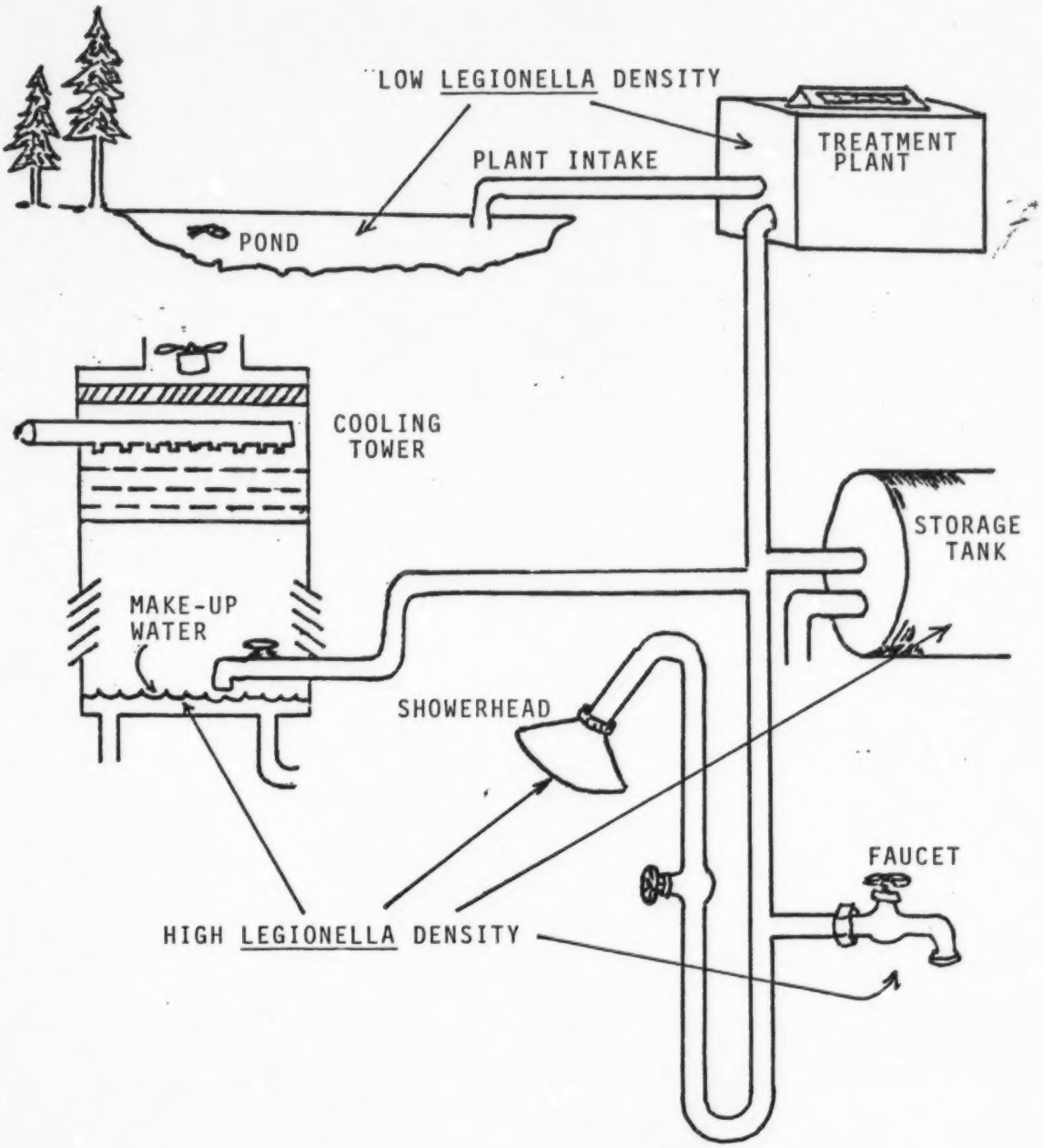


Figure 4.


LOW LEGIONELLA DENSITY
TREATMENT PLANT
PLANT INTAKE
POND
COOLING TOWER
STORAGE TANK
MAKE-UP WATER
SHOWERHEAD
FAUCET
HIGH LEGIONELLA DENSITY